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Terms	Documents
l7 and (in situ)	68

Database:
- US Patents Full-Text Database
- US Pre-Grant Publication Full-Text Database
- JPO Abstracts Database
- EPO Abstracts Database
- Derwent World Patents Index
- IBM Technical Disclosure Bulletins

Refine Search: `l7 and (in situ)` Clear

Search History

Today's Date: 6/14/2001

DB Name	Query	Hit Count	Set Name
USPT	l7 and (in situ)	68	L9
USPT	l7 and (in vivo)	171	L8
USPT	l6 and scfv	203	L7
USPT	l4 and antibody near5 fragment	5604	L6
USPT	l4 and antibody near5 fragment	5604	L5
USPT	libraries	37141	L4
USPT	library	37141	L3
USPT	libra?	42	L2
USPT	libra?5	0	L1

protein binding human **scFv** with affinities comparable to murine hybridomas can be produced without immunization.

L8 ANSWER 12 OF 12 CA COPYRIGHT 2001 ACS
ACCESSION NUMBER: 124:84393 CA
TITLE: Use of a novel mutagenesis strategy, optimized residue substitution, to decrease the off-rate of an anti-gp120 **antibody**
AUTHOR(S): Lewis, Craig M.; Hollis, Gregory F.; Mark, George E, III; Tung, Jwu-Sheng; Ludmerer, Steven W.
CORPORATE SOURCE: Merck Res. Laboratories, Rahway, NH, 07065, USA
SOURCE: Mol. Immunol. (1995), 32(14/15), 1065-72
CODEN: MOIMD5; ISSN: 0161-5890
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The authors have developed a novel strategy to decrease the **antibody**-antigen off-rate which the authors call optimized residue substitution. This strategy employs alanine substitution to first identify residues non-optimal for binding, as evidenced by a decrease in off-rate upon alanine replacement. These positions are then individually randomized to all amino acids, and the best replacement for each position detd. Finally, a construct which combines all optimized substitutions is generated and evaluated. The authors applied this strategy to the heavy chain CDR3 of P5Q, a **scFv antibody** which recognizes an epitope on the V3 loop of HIV gp120. The authors identified two amino acid substitutions that together decrease the off-rate by nearly ten-fold.
The contributions by the two substitutions were near additive, indicative of independent affects on binding. The authors suggest that this strategy can be generalized to strengthen protein:ligand and protein:protein interactions in other systems.

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FILE 'CA' ENTERED AT 13:07:23 ON 14 JUN 2001
L1 315821 S ANTIBO?
L2 0 S L1 AND SCFV C215 FRAGMENT
L3 0 S L1 AND SINGLE CHAIN C215 FRAGMENT
L4 1155 S L1 AND SCFV
L5 0 S L4 AND C215 FRAGMENT
L6 286 S L4 AND LIBRAR?
L7 0 S L6 AND EPITOPE EPITHELIAL GLYCOPROTEIN
L8 12 S L6 AND GLYCOPROTEIN

line. The immunotoxin is very stable at 37.degree.C, retaining 80% of its original activity after 24 h. Potent immunotoxins such as 3B3(Fv)-PE38 could be utilized in combination with multidrug cocktails that limit viral replication to help reduce viral reservoirs in patients with AIDS.

L8 ANSWER 6 OF 12 CA COPYRIGHT 2001 ACS
ACCESSION NUMBER: 129:158874 CA
TITLE: In situ identification of structures binding to target structures e.g. in vivo selection method for a phage **library**
INVENTOR(S): Brodin, Thomas; Tordsson, Jesper; Karlstrom, Pia Jasmine
PATENT ASSIGNEE(S): Pharmacia & Upjohn AB, Swed.
SOURCE: PCT Int. Appl., 44 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9834110	A1	19980806	WO 1998-SE83	19980121
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9858879	A1	19980825	AU 1998-58879	19980121
EP 975964	A1	20000202	EP 1998-902323	19980121
R: DE, DK, FR, GB, IT, SE, IE				
ZA 9800795	A	19980805	ZA 1998-795	19980130

PRIORITY APPLN. INFO.: SE 1997-291 19970131
WO 1998-SE83 19980121

AB The invention relates to a selection method and the products resulting from the method, according to which one or more binding structures against a target structure is obtained by means of a first **library** of one or more binding structures linked to genetic and/or other identifying information. The method comprises the steps of reacting a first **library** with the displayed target structure to bind some of the binding structures to the displayed target structure, sepg. the displayed target structure and bound binding structures from unbound binding structures, recovering bound or unbound binding structures, and amplifying bound or unbound binding structures to create a second enriched **library** of binding structures. Identified binding structures are directed to target structures which are displayed in vivo and/or in situ. By selection of an **antibody** phage **library** derived from a human melanoma-immunized primate using metastatic melanoma tissue sections, a clone was identified which stained melanoma cells in tissue sections and defined a cell surface antigen expressed in cultured human melanoma cells but not in human peripheral blood mononuclear cells.

L8 ANSWER 7 OF 12 CA COPYRIGHT 2001 ACS
ACCESSION NUMBER: 128:191344 CA
TITLE: Efficient selection of **scFv antibody** phage by adsorption to in situ expressed antigens in tissue sections

AUTHOR(S): Tordsson, Jesper; Abrahmsen, Lars; Kalland, Terje; Ljung, Catherine; Ingvar, Christian; Brodin, Thomas
CORPORATE SOURCE: Pharmacia and Upjohn AB Lund Research Center, Lund, S-220 07, Swed.
SOURCE: J. Immunol. Methods (1997), 210(1), 11-23
CODEN: JIMMBG; ISSN: 0022-1759
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The present report describes the development and application of an efficient method for the direct adsorption/selection of **antibody** phage using antigens expressed in situ in cryostat tissue sections. In a model system, **scFv** phage directed towards an epitope on the GA733-2 epithelial **glycoprotein** expressed in colorectal carcinoma tissue could be specifically enriched up to 1500-fold in single-pass expts. and a million fold after 3 rounds of selection. Enrichment efficacy was directly proportional to the fraction of antigen pos. area over the total area. Sufficient enrichment was achieved at an area fraction of <4%, thereby permitting the selection of **antibodies** to subpopulations of cells or to tissue substructures. The general usefulness of the method was demonstrated when a combinatorial **scFv antibody** phage **library** derived from melanoma immunized non-human primates was selected in tissue sections of metastatic melanoma. Individual **scFv antibodies** from enriched phage populations demonstrated different binding specificities, reflected in extracellular and cellular tissue staining patterns which included tumor cell surface reactivity. This method should be particularly useful for the identification of antigens which are only expressed during specific in vivo conditions, and overcomes a major limitation of currently used selection protocols.

102(a)

L8 ANSWER 8 OF 12 CA COPYRIGHT 2001 ACS
ACCESSION NUMBER: 127:306405 CA
TITLE: Phage-displayed and soluble mouse **scFv** fragments neutralize rabies virus
AUTHOR(S): Muller, Bruno H.; Lafay, Florence; Demangel, Caroline; Perrin, Pierre; Tordo, Noel; Flamand, Anne; Lafaye, Pierre; Guesdon, Jean-Luc
CORPORATE SOURCE: Lab. Predeveloppement des Sondes, Inst. Pasteur, Paris, 75724, Fr.
SOURCE: J. Virol. Methods (1997), 67(2), 221-233
CODEN: JVMEDH; ISSN: 0166-0934
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A phage-display technol. was used to produce a single-chain Fv **antibody** fragment (**scFv**) from the 30AA5 hybridoma secreting anti-**glycoprotein** monoclonal **antibody** (MAb) that neutralizes rabies virus. **ScFv** was constructed and then cloned for expression as a protein fusion with the g3p minor coat protein of filamentous phage. The display of **antibody** fragment on the phage surface allows its selection by affinity using an ELISA; the selected **scFv** fragment was produced in a sol. form secreted by E. coli. The DNA fragment was sequenced to define the germline gene family and the amino-acid subgroups of the heavy (VH) and light (VL) chain variable regions. The specificity characteristics and neutralization capacity of phage-displayed and sol. **scFv** fragments were identical to those of the parental 30AA5 MAb directed against antigenic site II of rabies **glycoprotein**. Phage-display technol. allows the prodn. of new **antibody** mol. forms able to neutralize the rabies virus specifically. The next step could be to engineer and produce

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multivalent and multispecific neutralizing **antibody** fragments for passive Ig therapy.

L8 ANSWER 9 OF 12 CA COPYRIGHT 2001 ACS
ACCESSION NUMBER: 125:84122 CA
TITLE: A CD66a-specific, activation-dependent epitope detected by recombinant human single chain fragments (svFvs) on CHO transfectants and activated granulocytes
AUTHOR(S): Jantscheff, Peter; Nagel, Gerhard; Thompson, John; Kleist, Sabine V.; Embleton, M. J.; Price, Michael R.; Grunert, Fritz
CORPORATE SOURCE: Inst. Immunobiology, Albert-Ludwigs-Univ. Freiburg, Germany
SOURCE: J. Leukocyte Biol. (1996), 59(6), 891-901
CODEN: JLBIE7; ISSN: 0741-5400
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Antibodies** to CD66 recognize at least five members (CD66a-3) of the carcinoembryonic antigen (CEA) family. Recombinant human single-chain Fv fragments (scFvs) that bind specifically to CD66a (biliary **glycoprotein**) were obtained from a naive human **scFv library**. The scFvs bound to the N-domain of Cd66a on Chinese hamster ovary (CHO) transfectants but did not bind to freshly isolated peripheral granulocytes or to dimethylsulfoxide-treated HL-60 cells. In contrast, scFvs bound well to granulocytes that were short-term activated with N-formyl-Met-Leu-PHe or phorbol 12-myristate 13-acetate and to human HL-60 cells that were treated with all-trans-retinoic acid to induce a granulocytic differentiation. Quantification of antigenic site showed that the activation-dependent CD66a epitopes were expressed on nearly all of the CD66a mols. on CHO-biliary **glycoprotein** transfectants, but they were detected only on a portion of the mols. on activated polymorphonuclear neutrophils and differentiated HL-60 cells. Binding of CD66a scFvs to their neoepitopes on prestimulated PMNs induced respiratory burst, suggesting that CD66a is capable of delivering transmembrane signals in these cells.

L8 ANSWER 10 OF 12 CA COPYRIGHT 2001 ACS
ACCESSION NUMBER: 124:257983 CA
TITLE: Identification of functional and structural amino-acid residues by parsimonious mutagenesis
AUTHOR(S): Schier, Robert; Balint, Robert F.; Mc, Call, Adrian; Apell, Gerald; Larrick, James W.; Marks, James D.
CORPORATE SOURCE: Departments of Anesthesia and Pharmaceutical Chemistry, University of California, San Francisco, San Francisco General Hospital, San Francisco, CA, 94110, USA
SOURCE: Gene (1996), 169(2), 147-55
CODEN: GENED6; ISSN: 0378-1119
DOCUMENT TYPE: Journal
LANGUAGE: English
AB For in vitro evolution of protein function, the authors previously proposed using parsimonious mutagenesis (PM), a technique where mutagenic oligodeoxynucleotides (oligo) are designed to minimize coding sequence redundancy and limit the no. of amino acid (aa) residues which do not retain parental structural features. For this work, PM was used to increase the affinity of C6.5, a human single-chain Fv (**scFv**) that binds the **glycoprotein** tumor antigen, c-erbB-2. A phage **antibody library** was created where 19 aa located in three of the heavy (H) and light (L) chain antigen-binding loops (L1, L3 and H2) were simultaneously mutated. After four rounds of selection, 50%

of **scFv** had a lower dissocn. rate const. (koff) than the parental **scFv**. Kd of these **scFv** ranged from twofold (Kd = 7.0 .times. 10-9 M) to sixfold (Kd = 2.4 .times. 10-9 M) lower than the parental **scFv** (Kd = 1.6 .times. 10-8 M). In higher affinity **scFv**, substitutions occurred at 10/19 of the positions, with 21/28 substitutions occurring at only four positions, two in H2, and one each in L1 and L3. Only the wild type (wt) aa was obsd. at 9/19 aa. Based on a model of C6.5, seven of the nine conserved aa have a structural role in the variable domain, either in maintaining the main chain conformation of the loop, or in packing on the H-chain variable domain. Two of the conserved aa are solvent exposed, suggesting they may play a crit. role in recognition. Thus, PM identified three types of aa: structural aa, functional aa which modulate affinity, and functional aa, which are crit. for recognition. Since the sequence space was not completely sampled, higher affinity **scFv** could be produced by subjecting functional aa which modulate affinity to a higher rate of mutation. Furthermore, PM could prove useful for modifying function in other proteins that belong to structurally related families.

L8 ANSWER 11 OF 12 CA COPYRIGHT 2001 ACS
ACCESSION NUMBER: 124:114968 CA
TITLE: Isolation of high-affinity monomeric human anti-c-erbB-2 single chain Fv using affinity-driven selection
AUTHOR(S): Schier, Robert; Bye, Jacqueline; Apell, Gerald; McCall, Adrian; Adams, Gregory P.; Malmqvist, Magnus; Weiner, Louis M.; Marks, James D.
CORPORATE SOURCE: Dep. Anesthesia Pharmaceutical Chem., Univ. California, San Francisco, CA, 94110, USA
SOURCE: J. Mol. Biol. (1996), 255(1), 28-43
CODEN: JMOBAK; ISSN: 0022-2836
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The use of **antibodies** to target tumor antigens has had limited success, partially due to the large size of IgG mols., difficulties in constructing smaller single chain Fv (**scFv**) **antibody** fragments, and immunogenicity of murine **antibodies**. These limitations can be overcome by selecting human **scFv** directly from non-immune or semi-synthetic phage **antibody libraries**; however, the affinities are typically too low for therapeutic application. For hapten antigens, higher-affinity **scFv** can be isolated from phage **antibody libraries** where the VH and VL genes of a binding **scFv** are replaced with repertoires of V genes (chain shuffling). The applicability of this approach to protein binding **scFv** is unknown. For this work, chain shuffling was used to increase the affinity of a non-immune human **scFv**, which binds the **glycoprotein** tumor antigen c-erbB-2 with an affinity of 1.6 .times. 10-8 M. The affinity of the parental **scFv** was increased sixfold (Kd = 2.5 .times. 10-9 M) by light-chain shuffling and fivefold (Kd = 3.1 .times. 10-9M) by heavy-chain shuffling, values comparable to those for **antibodies** against the same antigen produced by hybridomas. When selections were performed on antigen immobilized on polystyrene, spontaneously dimerizing **scFv** were isolated, the best of which had only a slightly lower Kd than wild type (Kd = 1.1 .times. 10-8 M). These **scFv** dimerize on phage and are preferentially selected as a result of increased avidity. Compared to **scFv** which formed only monomer, dimerizing **scFv** had mutations located at the VH-VL interface, suggesting that VH-VL complementarity dets. the extent of dimerization. Higher-affinity monomeric **scFv** were only obtained by selecting in soln. using limiting concns. of biotinylated antigen, followed by screening mutant **scFv** from bacterial periplasm by koff in a BIA-core. Using the proper selection and screening conditions,

L8 ANSWER 1 OF 12 CA COPYRIGHT 2001 ACS
ACCESSION NUMBER: 134:309536 CA
TITLE: Efficient generation of respiratory syncytial virus (RSV)-neutralizing human MoAbs via human peripheral blood lymphocyte (hu-PBL)-SCID mice and **scFv** phage display **libraries**
AUTHOR(S): Nguyen, H.; Hay, J.; Mazzulli, T.; Gallinger, S.; Sandhu, J.; Teng, Y.-T. A.; Hozumi, N.
CORPORATE SOURCE: Department of Laboratory Medicine and Pathobiology, Samuel Lunenfeld Research Institute, University of Toronto, Toronto, ON, Can.
SOURCE: Clin. Exp. Immunol. (2000), 122(1), 85-93
CODEN: CEXIAL; ISSN: 0009-9104
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB RSV is one of the major causes of pneumonia and bronchiolitis in infants and young children and is assocd. with high mortality. RSV neutralizing human **antibody** (hu-Ab) is known to mediate resistance to viral infection as well as to be an effective treatment for severe lower respiratory tract RSV infection. We have previously demonstrated that human primary and secondary immune responses can be established in severe combined immunodeficient mice engrafted with human peripheral blood lymphocytes (hu-PBL-SCID). By combining this animal model with the single-chain Fv **antibody** (**scFv**) phage display **library** technique, we were able to investigate further its clin. potential by generating a panel of human scFvs that exhibit both high F **glycoprotein** (RSV-F) binding affinities (.apprx.108 M-1) and strong neutralizing activities against RSV infection in vitro. Sequencing anal. of the randomly isolated anti-RSV-F **scFv** clones revealed that they were derived from different VH families with mutations in the complementarity-detg. region 1 (CDR1). The results suggest that: (i) RSV-F-specific human immune responses and affinity maturation can be induced in hu-PBL-SCID mice; and (ii) this approach can be applied to generate large nos. of human scFvs with therapeutic potential. Despite the fact that hu-PBL-SCID mouse and human **scFv** phage display **library** have individually been established, our approach contributes a simple and significant step toward the generalization of antigen-specific human monoclonal **antibody** (hu-MoAb) prodn. and their clin. applications.
REFERENCE COUNT: 38
REFERENCE(S): (1) Albert, S; J Immunol 1997, V159, P1393 CA
(2) Barbas, C; Proc Natl Acad Sci USA 1992, V89, P10164 CA
(3) Beeler, J; J Virol 1989, V63, P2941 CA
(4) Bird, R; Tibtech 1991, V9, P132 CA
(5) Bocher, W; Immunol 1999, V96, P634 CA
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 2 OF 12 CA COPYRIGHT 2001 ACS
ACCESSION NUMBER: 132:346624 CA
TITLE: Variable heavy chain and variable light chain regions of **antibodies** to human platelet **glycoprotein** Ib alpha
INVENTOR(S): Miller, Jonathan L.
PATENT ASSIGNEE(S): USA

SOURCE: PCT Int. Appl., 89 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000026667	A1	20000511	WO 1999-US25495	19991029
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1051620	A1	20001115	EP 1999-971513	19991029
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				

PRIORITY APPLN. INFO.: US 1998-106275 P 19981030
WO 1999-US25495 W 19991029

AB The present invention is directed to a method of selecting a clone that binds to human platelet **glycoprotein** Ib alpha using a human variable heavy chain and variable light chain Ig **library**. The invention is further directed to isolated nucleic acid mols. encoding a variable heavy chain or variable light chain region of an **antibody**, wherein the **antibody** binds to human platelet **glycoprotein** Ib alpha and inhibits aggregation of platelets. Expression vectors and host cells comprising the nucleic acid mols. are also provided, as well as methods for producing the variable heavy chain or the variable light chain region. An isolated variable heavy chain or variable light chain region of an **antibody**, wherein the **antibody** binds to human platelet **glycoprotein** Ib alpha and inhibits aggregation of platelets, is also provided. An **antibody** comprising the variable heavy chain or variable light chain regions is provided, as is a compn. comprising the **antibody** and a carrier. The subject invention further provides a method of inhibiting aggregation of platelets, as well as a method of binding human platelet **glycoprotein** Ib alpha. A method of selecting a variable heavy chain or variable light chain region of an **antibody** is also provided.

REFERENCE COUNT: 4
REFERENCE(S):
(1) Griffiths; EMBO J 1994, V13(14), P3245 CA
(2) Konkle; The Journal of Biological Chemistry 1990, V265(32), P19833 CA
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(4) Nissim; EMBO J 1994, V13(3), P692 MEDLINE

L8 ANSWER 3 OF 12 CA COPYRIGHT 2001 ACS
ACCESSION NUMBER: 132:320708 CA
TITLE: Guided Selection of a Pan Carcinoma Specific **Antibody** Reveals Similar Binding Characteristics yet Structural Divergence Between the Original Murine **Antibody** and its Human Equivalent
AUTHOR(S): Beiboer, Sigrid H. W.; Reurs, Anneke; Roovers, Rob C.; Arends, Jan-Willem; Whitelegg, Nick R.; Rees, Anthony R.; Hoogenboom, Hennie R.
CORPORATE SOURCE: Research Institute Growth and Development, Department of Pathology, Maastricht University, Maastricht, Neth.

SOURCE: J. Mol. Biol. (2000), 296(3), 833-849
CODEN: JMOBAK; ISSN: 0022-283[redacted]
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Antibody** engineering provides an excellent tool for the generation of human immunotherapeutics for the targeted treatment of solid tumors. The authors have engineered and selected a completely human **antibody** to epithelial **glycoprotein**-2 (EGP-2), a transmembrane **glycoprotein** present on virtually all human simple epithelia and abundantly expressed on a variety of human carcinomas. The authors chose to use the procedure of "guided selection" to rebuild a high-affinity murine **antibody** into a human **antibody**, using two consecutive rounds of variable domain shuffling and phage **library** selection. As a starting **antibody**, the murine **antibody** MOC-31 was used. After the first round of guided selection, where the VH of MOC-31 was combined in Fab format with a human VLCL **library**, a small panel of human light chains was identified, originating from a segment of the V.kappa.III family, whereas the MOC-31 VL is more homologous to the V.kappa.II family. Nevertheless, one of the chimeric Fabs, C3, displayed an off-rate similar to MOC-31 **scFv**. Combining the VL of C3 with a human VH **library**, while retaining the VH CDR3 of MOC-31, clones were selected using human VH genes originating from the rarely used VH7 family. The best clone, 9E, shows over 13 amino acid mutations from the germline sequence, has an off-rate comparable to the original **antibody** and specifically binds to the "MOC-31"-epitope on EGP-2 in specificity and competition ELISA, FACS anal. and immunohistochem. In both VL and VH of **antibody** 9E, three germline mutations were found creating the MOC-31 homolog residue. Structural modeling of both murine and human **antibodies** reveals that one of the germline mutations, 53Y in VH CDR2, is likely to be involved in antigen binding. The authors conclude that, although they may bind the same epitope and have similar binding affinity to the antigen as the original murine **antibody**, human **antibodies** derived by guided selection unlike CDR-grafted **antibodies**, may retain only some of the original key elements of the binding site chem. The selected human anti-EGP-2 **antibody** will be a suitable reagent for tumor targeting. (c) 2000 Academic Press.

REFERENCE COUNT: 50
REFERENCE(S):
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(6) Chothia, C; Nature 1989, V342, P877 CA
(7) Connolly, M; J Appl Crystallog 1983, V16, P548 CA
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 4 OF 12 CA COPYRIGHT 2001 ACS
ACCESSION NUMBER: 131:115025 CA
TITLE: Functional expression in bacteria and plants of an **scFv antibody** fragment against tospoviruses
AUTHOR(S): Franconi, Rosella; Roggero, Piero; Pirazzi, Paola; Arias, Francisco Javier; Desiderio, Angiola; Bitti, Orsola; Pashkoulov, Dimitre; Mattei, Benedetta; Bracci, Luisa; Masenga, Vera; Milne, Robert Geoffrey; Benvenuto, Eugenio
CORPORATE SOURCE: ENEA, Dipartimento Innovazione, Divisione Biotecnologie e Agricoltura, Rome, Italy
SOURCE: Immunotechnology (1999), 4(3,4), 189-201
CODEN: IOTEER; ISSN: 1380-2933
PUBLISHER: Elsevier Science Ireland Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Recombinant **antibodies** expressed in plants ("plantibodies"), directed against crucial antigens and addressed to the right cell compartment, may be able to protect against viral diseases. Moreover, **antibody** fragments produced in bacteria or plants may provide low cost reagents for immunodiagnosis. In an attempt to develop genetic immunization against tomato spotted wilt tospovirus (TSWV), we engineered an **scFv** fragment starting from a monoclonal **antibody** (mAb) able to recognize an epitope of the **glycoprotein** G1 conserved among a large no. of tospoviruses. After establishing functional expression in bacteria, we aimed to drive expression of this mol. in the secretory pathway of plants. An **antibody** phage display expression system was used to isolate the correct VH and VL binding regions from the hybridoma secreting the original mAb. To assess functional expression in plant, we first used an epichromosomal expression vector derived from potato virus X (PVX). In this vector the **scFv** gene was cloned to produce a cytosolic or a secretory protein. For secretion, the signal sequence derived from the polygalacturonase-inhibiting protein (PGIP) of Phaseolus vulgaris was used. Subsequently, the gene encoding the secretory **scFv**, was used to transform Nicotiana benthamiana plants. High expression levels of fully active mol. were obtained in Escherichia coli. The engineered mol. retained the binding specificity and dissocn. rate const. (koff) of the cognate monoclonal **antibody**. Both PVX-infected and transformed plants expressed fully functional **scFv** mols. in the secretory pathway. This engineered **scFv** may be valuable.

REFERENCE COUNT: 43

REFERENCE(S):
(2) Adam, G; Arch Virol 1993, V130, P237 CA
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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 5 OF 12 CA COPYRIGHT 2001 ACS

ACCESSION NUMBER: 129:229342 CA

TITLE: Specific killing of HIV-infected lymphocytes by a recombinant immunotoxin directed against the HIV-1 envelope **glycoprotein**

AUTHOR(S): Bera, Tapan K.; Kennedy, Paul E.; Berger, Edward A.; Barbas, Carlos F., III; Pastan, Ira

CORPORATE SOURCE: Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD, 20892-4255, USA

SOURCE: Mol. Med. (N. Y.) (1998), 4(6), 384-391
CODEN: MOMEF3; ISSN: 1076-1551

PUBLISHER: Springer-Verlag New York Inc.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB 3B3 is a high-affinity anti-gp 120 **antibody** that neutralizes a wide range of primary and lab. isolates of HIV-1. The parental **antibody** was isolated from a combinatorial phage display **library** constructed from bone marrow RNA of an HIV-infected individual. We have generated a highly active immunotoxin using the 3B3 single-chain Fv (**scFv**) which can specifically kill lymphocytes infected by HIV-1. We used recombinant DNA technol. to clone the Fv fragment of 3B3 and produce a single-chain Fv (**scFv**). 3B3 **scFv** was then fused to a truncated version of Pseudomonas exotoxin A (PE38), giving rise to a recombinant immunotoxin 3B3(Fv)-PE38 that was expressed in E. coli and purified to near homogeneity. 3B3(Fv)-PE38 binds with the same affinity as the parental Fab **antibody** to the MN strain of gp 120. The immunotoxin specifically kills a gp 120-expressing transfected cell line and a chronically HIV-infected lymphocytic cell